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A Proposed Method for the Measurement of the Respiratory Exchange and Energy Expenditure of Grazing Animals [1/]

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The amount of energy expended by a grazing animal has been the subject of much speculation, but the lack of a suitable experimental method has prevented any precise measurement of this factor. A knowledge of the energy expenditure of grazing animals would facilitate the evaluation of the nutritive value of pastures, as well as make it possible to obtain more accurate estimates of the energy requirements of animals under these circumstances.

A number of techniques, including direct calorimetry, indirect open-circuit calorimetry, closed-circuit respiration chambers, closed-circuit spirometers, or long-term feeding trials in combination with body composition data, have been effectively used for energy metabolism studies with large animals. However, each of these methods has serious limitations for studies involving grazing animals. A procedure has been devised to determine the oxygen consumption, carbon dioxide production and energy expenditure of large animals for extended periods of time without restricting or confining them. The energy expenditure of activities such as walking, grazing, ruminating, chewing, lactating, standing and lying may be measured. The method also makes it possible to conduct a complete energy and carbon nitrogen balance on animals under selected environmental conditions. The gases resulting from fermentation in the gastrointestinal tract as opposed to animal tissue respiration may be determined by using this procedure in conjunction with a respiration chamber. The amounts of combustible gases which are absorbed and eliminated through the respiratory tract are also determinable. The proposed technique involves tracheal transection for tracheal intubation and a light weight portable dry gas meter with a proportional aliquoting mechanism.

Tracheal cannulae were used quite extensively by some of the early German workers to study energy expenditure in short term experiments. In their experiments it was not possible for the animal to have freedom of movement except possibly on a treadmill because of the excessive weight of the gas meters and the elaborate fragile aliquoting devices. The classical work of Zuntz *et al.* (1898), Zuntz and Lehmann (1889) and Lehmann et al. (1894) on the metabolism of the horse during rest and work was performed primarily by means of tracheotomized horses.

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Similar methods were used successfully by Hagemann (1899), Hagemann and Karpow (1906) and Ustjanzew (1911) to study energy expenditure of tracheotomized sheep. Cattle energy metabolism studies were conducted by Pächtner (1909), Dahm (1910), Heide et al. (1913), Klein (1912, 1915) and Möllgaard and Andersen (1917) by making use of a tracheal cannula. Klein (1915) compared the closed circuit (Regnault-Reiset), open-circuit (Pettenkofer-Tigerstedt) and tracheal cannula (Zuntz) methods using one animal and found close agreement for O_2 consumption. However, the CO_2 and CH_4 production differed appreciably and from his data he estimated the amounts of these gases which were produced by fermentation and excreted through the lungs, skin and intestinal tract. While the methods used by Klein were quite empirical, with refinements many questions could be resolved by the techniques he employed.

More recently New Zealand workers, Cresswell (1957) and Webster and Cresswell (1957), reported the use of tracheotomized goats with a closed circuit human basal metabolism apparatus to study climatological, nutritional and energy metabolism studies with ruminants. Lack of portability is the major limitation of their method.

EXPERIMENTAL TECHNIQUES

The method selected is based on the open-circuit indirect calorimetry principle in which the animal inspires outdoor air and the expired air is measured, aliquoted and analyzed for CO_2, O_2 and N_2. The important differences between this procedure and those used by previous workers are that this scheme allows almost complete freedom of movement of the animal, portability and trials of greater length. A rugged, light weight portable dry gas meter with a continuous proportional aliquoting device designed for human energy expenditure research by Müller and Franz (1952) was adapted to measure larger volumes and collect a smaller aliquot. The meter is attached to the animal by means of a harness and expired air is measured by direct connection of the meter to a tracheal cannula. The aliquot of expired air is collected in a 100 liter Douglas bag which is also harnessed to the animal.

Tracheal transection was performed on two Jersey cows as described by Colvin et al. (1957). Complete transection with suturing to the skin gave a spread of three to five inches between the exposed openings of the trachea. These openings were joined by a piece of flexible plastic (Tygon or Cobon) tubing when the animal was not on trial. A 12" length of tubing with 1/8" wall thickness and as large in diameter as would fit into the trachea (1" I.D. for 300-400 Kg. animal) has been used. A piece of 1/2" webbing tied around the tube and then around the animal's neck served to hold it in place. The animals were checked daily to make sure the tube stayed in place. One animal was run with the milking herd for exercise and kept in a stanchion for 6 weeks without the tube having to be adjusted. Mucus accumulated in the plastic tube and tended to gradually constrict the opening, so it should be removed and cleaned at least once a month. The tube should never be removed from the trachea for more than a short period of time because the opening immediately begins to constrict and the animal would asphyxiate. Besides preventing constriction, the plastic tube makes it possible for the animal to smell and communicate (moo).

To obtain a measure of respiratory exchange the connecting tube is removed and a catheter of the same flexible material is inserted into the posterior portion of the trachea. This catheter has an inflatable cuff (Sanders large single wall) to give a gas tight seal in the trachea. The exposed end of the catheter was built up with rings of the same plastic tubing to match the valve connection size. Cyclohexanone was used as a bonding agent for this flexible tubing.

The plastic respiratory valve supplied as an accessory with the Müller-Franz meter was too small for use with dairy cattle, so a larger valve identical in design was built. The valve body was machined from 2 3/4" solid acrylic resin plastic (Lucite or Plexiglas) giving a 2 5/16" inside diameter. The valve seat for the expiratory valve was machined as an integral part of the body. The inspiratory, tracheal and expiratory openings were 1 1/2" inside diameter. Methylene chloride was used as a bonding agent for acrylic resin plastic. The mica valves were too fragile, so they were replaced with valves made of sheet polystyrene as suggested by workers at the Army Medical Nutrition Laboratory (1954).

Commercial vacuum cleaner (1 1/2" I. D.) hose coated with liquid neoprene has been used to transfer the expired air from the valve to the meter. This tube is somewhat stiffer than smaller conventional tubing used for this purpose and another more flexible tube may be more desirable.

Müller-Franz portable dry gas meters with extremely low resistance (10 mm water at 20 L per min.) were used to measure and aliquot the expired gas. The standard model as used in human studies was modified to give a capacity of 50 to 100 liters per minute, aliquot settings of 0.1 and 0.2%, 7 digit readings in liters and a 1.5 inch intake. This enlarged meter retains a precision of ±1.5%. The Army Medical Nutrition Laboratory (1954) has indicated that the delivered aliquot may not be exactly equal to the setting, although the aliquot was a constant proportion of the expired gas. A calibration curve was supplied with each instrument. A change in the meter calibration was noted by the Army Medical Nutrition Laboratory (1954) when the position of the aliquot selector knob was changed.

In order to collect data over periods of 6 to 12 hours a 100 liter Douglas bag (Davol) must be substituted for the small aliquot sampling bag. The diffusion loss of CO_2 for 12 hour storage of 2 full bags of expired air was about 5%. The increase of O_2 was less than 1%. Coating one bag with 5 brushed coats (3 qts.) of liquid neoprene reduced the diffusion of both gases to within limits of acceptable precision for gas analysis by the Haldane method. With continuous filling for the 12 hour period these losses would be approximately half this value. The neoprene coating decreased the flexibility of the bags and therefore increased the difficulty in rinsing the bags at the start of a trial. Larger corrections are required to adjust for this increase in functional dead space.

The bag and the Müller-Franz meter as well as urine and feces bags were attached to a Gorski et al. (1957) harness. Urine nitrogen values are required. Feces bags may be desirable to eliminate the error of grab sampling. The total weight of the equipment, including harness and all the apparatus is 13.3 Kg. The weight of the equipment used only for respiratory exchange studies is 7.1 Kg.

Two methods of calculation of energy are possible. A short method proposed by Weir (1949) requires knowledge of the volume of exhaled air, percentage oxygen in expired air and percent protein in the diet. The longer conventional method requires oxygen consumption, CO_2 production, and urinary nitrogen. The accuracy of the shorter method for studies with ruminants has not been determined, so the latter, more laborious method is the method of choice pending further investigations. The examples in table 1 are given to demonstrate the methods of calculating the results.

Table 1 - Example using data on 300 Kg. cow for 24 hours.

Conventional method

1.	gm.	urinary nitrogen excreted	74.67
2.	l.	oxygen consumption by protein (1x5.94)	444
3.	l.	carbon-dioxide produced by protein (1x4.76)	355
4.	Cals.	equivalent of protein (1x26.51)	1980
5.	l.	total oxygen consumed	874
6.	l.	non-protein oxygen consumed (5-2)	430
7.	l.	total carbon dioxide produced	691
8.	l.	non-protein carbon dioxide produced (7-3)	336
9.	R.Q.	non-protein (8/6)	.78
10.	Cals.	non-protein (6x4.776)	2054
11.	Cals.	total (4+10)	4034

Short method of Weir

$$\text{Cals.} = \frac{(O_i - O_e)0.0504}{1 + 0.082\ p} \times \text{liters expired air}$$

where O_i = percent oxygen in inspired air
O_e = percent oxygen in expired air
p = decimal fraction protein in diet

$$= \frac{(20.93 - 17.88)\ 0.0504}{1 + 0.082\ (.15)}$$

$= 4164$

Some limitations to the use of this technique have been observed. The primary one is the effect of the operation on the animal. Previous workers have reported no ill effects over long periods of time, but the animals used in these studies coughed periodically and occasionally the respiratory valve became obstructed with mucus. This interfered with its efficient operation. The conditions under which this apparatus may be used in practice are not as well controlled as when a respiration chamber is used. However, the greater versatility and ease of conducting experiments under a wide variety of conditions help to compensate for any loss in precision. The method of choice would depend on the objectives of the experiment.

Some modifications of apparatus have been considered for incorporation into this method. Perkins (1954) described vinyl plastic Douglas bags which were much lighter and had lower functional dead space than the conventional ones and they may be used to advantage. However, the diffusion rate reported was more than that observed with the neoprene-coated Douglas bags. The Müller-Franz meter may be replaced with a light weight integrating motor pneumotachograph as designed by Fletcher and Wolff (1954). This flowmeter had a constant low resistance even at high rates of gas flow, but its construction was of Plexiglas and may not be as durable as the metal meters.

A list of sources of supplies used will be furnished upon request.

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